TITLE

REAL TIME QUANTITATIVE PCR WITH INTERCALATING DYE FOR SINGLE AND MULTIPLEX TARGET DNA

ABSTRACT

The PCR-based, dsDNA quantification method monitors the fluorescence of a target, whose melting characteristics is predetermined, during each amplification cycle at selected time-points. Fluorescence is measured immediately after the annealing phase (F_E at T_E), immediately below (F_{MS} at T_{MS}) and above (F_{ME} at T_{ME}) the melting of the target/amplicon. A change in slope from a baseline slope ($S_B = -(F_{MS} - F_E)/(T_{MS} - T_E)$) to a melting phase slope ($S_M = -(F_{ME} - F_{MS})/(T_{ME} - T_{MS})$) indicates a specific amplification. The number of amplification cycles (C_T) it takes for the quantity ($S_M - S_B$) to become greater than zero correlates with the starting concentration of the target (C). The concentration of the target in a sample is determined by comparing the value of C_T for the sample with a standard curve. By selecting targets with distinguishable melting curve characteristics, multiple targets can be simultaneously detected.